procedure for isolating mitochondria was being calibrated to make its results correspond to those obtained by related techniques. Moreover, the fit with the plausible theory that oxidative phosphorylation was localized in the mitochondrion was a central consideration in evaluating the technique. The procedure for developing the evidence certainly was not independent of the theoretical claim it was supposed to support. Clearly, the technique was being calibrated by existing techniques and evaluated by its ability to produce evidence that fit a plausible theory.

Centrifugation Regimes

Once material is prepared, it is ready to be placed into the centrifuge and spun. Many questions of procedure remained, however. How fast should the contents be spun, and for how long? For Bensley and Hoerr, who started with the objective of isolating mitochondria, the strategy was pretty straightforward: centrifuge long enough to separate what seemed to be a reasonably pure mitochondrial preparation. However, what came to be the dominant approach involved the separation of four different fractions. This involved successive centrifugation runs in which the sediment at each stage was removed and the remaining unsedimented material (called the *supernatant*) was subjected to centrifugation at a yet higher speed (see Figure 4.3).[12] Why this procedure? Through it, as we will see in the next chapter, Claude isolated what appeared to be chemically distinct components that could be linked to four different components of living cells: the nucleus (in the sediment separated out by the first centrifugation run on the original homogenate), mitochondria (in the sediment of the second and third runs), microsomes (in the sediment of the fourth run), and cell sap or cytosol (soluble protoplasmic material remaining in the supernatant after the fourth run).

[12] The following is Claude's description of his procedure: "The suspension was immediately centrifuged for one minute at 2000 r.p.m. in a horizontal centrifuge. This step was found to remove most of the liver fragments, the cells which had remained intact, the free nuclei and the red corpuscles. The supernatant fluid, or extract proper, which contained practically all the organic components equal to, or smaller than, three μ diameter, was spun at 18,000 r.p.m. in the high speed centrifuge, for exactly five minutes. At that speed, a five minute run was sufficient to bring down practically all the large secretory granules. The sediment was saved for further purification in the centrifuge. The small particles, which had remained in the supernate, were sedimented by a long run purified in the higher speed centrifuge, the 'long run' in this case being five minutes centrifugation at 18,000 × gravity. The procedure consisted in suspending the material in water and sedimenting it again at high speed, four times in succession" (1941, p. 267).